

# A new genus of non-bloodsucking aquatic cave leeches in the family Salifidae Johansson, 1909 (Erpobdelliformes) from the Wuling Mountains of Central China

Yan Tang<sup>1</sup>, Zhixiao Liu<sup>1</sup>, Haiyang Xiang<sup>1</sup>, Linbei Li<sup>1</sup>, Shi Li<sup>1</sup>, Yazhen Zou<sup>1</sup>, Takafumi Nakano<sup>2</sup>

<sup>1</sup> College of Biology and Environmental Sciences, Jishou University, Jishou 416000, China

<sup>2</sup> Department of Zoology, Graduate School of Science, Kyoto University, Kyoto 606-8502, Japan

<https://zoobank.org/CD1C4713-2787-439A-9202-EFD6245C9C7F>

Corresponding authors: Zhixiao Liu (zxliu1965@163.com); Takafumi Nakano (nakano@zoo.zool.kyoto-u.ac.jp)

Academic editor: Pavel Stoev ♦ Received 11 March 2025 ♦ Accepted 27 May 2025 ♦ Published 20 June 2025

## Abstract

This paper describes a species of non-bloodsucking aquatic leeches distributed in Shibadong Cave, Huayuan County, Xiangxi Tujia and Miao Autonomous Prefecture of Hunan Province, belonging to the Wuling Mountains of central China. Based on morphological examination, mitochondrial DNA COI gene sequencing, and phylogenetic analysis, the leech was identified as a new genus in the family Salifidae and named *Shibabdella* **gen. nov.** after its collecting locality, “Shibadong Cave,” with the type species named *Shibabdella wulingensis* **sp. nov.** The genetic distances between this new genus and other genera in the family Salifidae ranged from 19.01% to 23.75% based on COI gene sequence alignment. Morphologically, the main distinguishing features are as follows: one complete somite consists of nine annuli; the pharynx appears tubular, reaching XIV  $c_1$ – $c_4$ , with three myognaths anteriorly; accessory copulatory pores are absent; male and female gonopores are located in the ventral center and annular furrow of somite XII  $c_2/c_3$  (57<sup>th</sup>/58<sup>th</sup> annuli) and somite XII  $c_{11}/c_{12}$  (63<sup>rd</sup>/64<sup>th</sup> annuli), respectively, separated by six annuli. The atrium appears spherical in shape and lacks a pre-atrial loop. Ovisacs are strip-like and extend to the middle of somite XXI, then fold in the opposite direction.

## Key Words

Hirudinea, molecular phylogeny, new genus, Salifidae, *Shibabdella*, Wuling Mountains

## Introduction

Leech is a general term for the class *Hirudinea*, which is distinguished from other annelid taxa by the development of suckers at both ends of the body (Yang 1996). The monograph *Leech Biology and Behavior* by Sawyer (1986) provided a systematic and comprehensive study of the global literature on leeches in terms of anatomy and physiology, taxonomic distribution, and behavioral ecology. Since then, many researchers have published a number of new species and their respective views on the taxonomy of leeches, and more than 900 species of leeches are known globally (Magalhães et al. 2021). In the 1970s, the investigation of leech resources

by Chinese scholars vigorously promoted the study of leech classification in China (Song 1974; Song et al. 1977; Song and Feng 1978; Liu 1984a, b). Another monograph, *The Fauna of Hirudinea in China* by Yang (1996), introduced in detail the research history of *Hirudinea* from China as well as the biological and ecological characteristics of various groups of *Hirudinea*. So far, the leeches recorded from China include two subclasses, three orders, nine families, 38 genera, and 125 species (Yang et al. 2009; Liu et al. 2011; Nakano and Lai 2012; Nakano and Lai 2017; Huang et al. 2019; Wang et al. 2022; Li et al. 2025; Yin et al. 2025). However, due to the vast area of China, there is a lack of investigation and scientific data on leeches in most

Chinese regions. Therefore, research prospects remain for leech taxonomy and diversity in China.

Depending on their habitats, leeches are roughly categorized as “aquatic leeches” and “land leeches.” The vast majority of aquatic leeches live in freshwater, while most land leeches inhabit moist mountain forests (Sawyer 1986; Liu 2021). Among the terrestrial habitats that leeches can inhabit, karstic caves are subterranean spaces formed by the evolution of the Earth’s surface—special ecosystems that differ greatly from other terrestrial environments and contain many unique troglobites (Liu 2021). *Sinospelaeobdella* is a newly named genus of troglobitic leech that inhabits the ceiling of caves and specializes in feeding on the blood of cave-dwelling bats. Three species are currently known: *Sinospelaeobdella wulingensis*, *S. cavatuses*, and *S. jiangxiensis* (Yang et al. 2009; Huang et al. 2019; Liu 2021; Seesamut et al. 2023; Li et al. 2025). Another troglobitic species, *Chtonobdella jawarerenensis*, distributed in the Southern Hemisphere, belongs to the duognathous group. However, this species appears to have behavioral habits similar to *Sinospelaeobdella*, a trignathous group in the family Haemadipsidae. For example, both species feed on the blood of bats (Ewers 1974; Tessler et al. 2016). All other leeches found in karstic caves live in water and are mostly from the erpobdelliform families Erpobdellidae and Salifidae (Matsumoto 1976; Sawyer 1986; Sket et al. 2001; Yang et al. 2009).

Species of the family Salifidae occupy aquatic, semi-aquatic, or terrestrial habitats. However, they mostly swallow worms or insect larvae and are distinguished from other families by the presence of three pharyngeal ridges, each bearing one to two columnar stylets at its anterior end (Sawyer 1986; Yang 1996). Currently, six genera belong to Salifidae: *Salifa*, *Barbronia*, *Odontobdella*, *Sinodontobdella*, *Mimobdella*, and *Dineta*. Of these, *Salifa* and *Barbronia* are broadly distributed; *Mimobdella* lives in Japan, Sundaland, and China; *Odontobdella* inhabits the Sino-Japanese region and India; and *Sinodontobdella* is endemic to China (Sawyer 1986; Nesemann 1995; Yang 1996; Nakano and Nguyen 2015; Nakano et al. 2018; Yin et al. 2025). Except for *Dineta*, which is distributed in the Southern Hemisphere, the other five genera have been found in China.

The Wuling Mountains, one of the global biodiversity hotspots, are located in central China, at the intersection of the Southwest, South China, and Central China zoogeographical regions (Liu 2021). The Xiangxi Tujia and Miao Nationality Autonomous Prefecture is located in the hinterland of the Wuling Mountains, a preferred area for the investigation and study of cave animal diversity because of its well-developed karstic landscapes and numerous karstic caves (Huang et al. 2019; Liu 2021). Since July 2024, we have repeatedly collected a species of leech in the perennial water on the ground in Shibadong Cave of Shibadong Village, Huayuan County, Xiangxi Prefecture. By comparative examination of morphological features and phylogenetic analysis of the mitochondrial

cytochrome c oxidase subunit I (COI) gene, these leech specimens are new to science and are classified as a new genus in the family Salifidae.

## Materials and methods

### Specimen collection

Live leeches were carefully searched for in waters containing clay, mud, sand, and stones on the ground in Shibadong Cave. When the target animals were found, they were transferred into plastic bottles containing an appropriate amount of water and clay from their habitats by using tweezers and soft-bristle brushes. The mouths of the bottles were then sealed with two layers of gauze and placed in a backpack kept in darkness and at a suitable temperature to maintain normal living conditions. After the collected live specimens were brought back to the laboratory, they were placed in bottles with appropriate amounts of habitat water and clay in an artificial climate incubator (model: ZRG-350B, temperature setting: 18 °C).

### Preparation and preservation of specimens

Live specimens were placed in fresh water while anhydrous ethanol was gradually added dropwise until the alcohol concentration reached about 20%. The specimens were then immersed for 25 minutes. When the specimens’ bodies were fully stretched and no longer responded to external physical stimuli, they were removed from the solution, the mucus on their surface was gently wiped off, and their surface was washed with water to remove dirt. The specimens were then shaped to appear naturally straight. They were sealed and stored in 4% paraformaldehyde solution for morphological observation. Some live specimens were washed directly with water and preserved in 95% ethanol solution for molecular identification. The specimens are stored in the Animal Collection of the College of Biology and Environmental Sciences, Jishou University, and the Hunan Wuling Mountain Biological Science Museum, under the numbers HNHY01–HNHY11.

### Morphological observation

A digital vernier caliper (accuracy 0.01 mm) was used to measure body length, maximum body width, head width, and diameters of the oral and caudal suckers. Some morphological characteristics, such as the number of somites, annuli, and eyespots, as well as the locations of gonopores and the number of annuli spaced between them, were observed using a stereomicroscope (LEICA M205 C). Afterward, the specimens were dissected to observe the structure and distribution of their digestive and

reproductive systems, and the results were compared with relevant literature (Nesemann 1995; Yang 1996; Yang et al. 2009; Nakano and Nguyen 2015; Nakano et al. 2018; Huang et al. 2019).

DNA extraction, amplification, and sequencing

A small piece of muscle from the tail suckers was cut (to avoid contamination of DNA from other organisms in the crop and intestine) and stored in anhydrous ethanol. These tissues were then sent to Sangon Biotech (Shanghai) Co., Ltd. for DNA extraction using the Ezup Column Genomic DNA Extraction Kit for Animal Tissues (B518251). The mitochondrial DNA (mtDNA) COI gene was amplified using the universal primer set LCO1490/HCO2198 (Folmer et al. 1994). The polymerase chain reaction (PCR) conditions were as follows: pre-denaturation at 95 °C for 5 min; denaturation at 94 °C for 30 s; annealing at 63 °C for 30 s; extension at 72 °C for 30 s (10 cycles); then denaturation at 95 °C for 30 s; annealing at 58 °C for 30 s; extension at 72 °C for 30 s (30 cycles); with a final repair and extension step at 72 °C for 10 min. The amplified PCR products were detected and purified for sequencing.

Data processing and analysis

Morphological data obtained were counted and analyzed using EXCEL 2025 (Microsoft 365). DNA sequences of new specimens from this study were uploaded to the National Center for Biotechnology Information (NCBI) to run the BLAST program, then combined with some highly similar sequences for phylogenetic tree construction and genetic distance calculation in MEGA 12.0 (Sudhir et al. 2024) (Table 1). AF116023 is from Apakupakul et al. (1999); DQ235595 and DQ235598 are from Ocegüera-Figueroa et al. (2005); AY786455 is from Siddall et al. (2007); GQ368760 is from Phillips and Siddall (2009); AB675016 is from Nakano et al. (2011); HQ336340, HQ336341, HQ336342, HQ336343, and HQ336346 are from Ocegüera-Figueroa et al. (2011); AB679654, AB679656, AB679658, AB679688, and AB679704 are from Nakano (2012); LC029428, LC029429, LC029430, and LC029431 are from Nakano and Nguyen (2015); LC090771 is from Nakano and Meyer-Rochow (2016); LC184559 and LC184562 are from Nakano (2017); PP510626 and PP510627 are from Yin et al. (2025). In MEGA 12.0, all sequences were first aligned using MUSCLE. Fragments with many gaps were clipped, and high-consistency fragments were retained to reduce data bias caused by differences in sequence length. The phylogenetic tree was constructed using maximum likelihood (ML), followed immediately by standard bootstrap analysis with 1000 replicates. New sequences were deposited in the International Nucleotide Sequence Database (INSd) through GenBank under the accession numbers PQ860767, PQ860768, and PQ860769.

**Table 1.** Accession numbers and basic information of mtDNA COI gene sequences in GenBank used to construct the phylogenetic tree (*Shibabdella wulingensis* PQ860767, PQ860768, and PQ860769 are the accession numbers of the aquatic leech specimens in this study, respectively).

Name of species	Locality	GenBank Accession Number	Source
Ingroup			
<i>Shibabdella wulingensis</i>	China	PQ860767	INSd
<i>Shibabdella wulingensis</i>	China	PQ860768	INSd
<i>Shibabdella wulingensis</i>	China	PQ860769	INSd
<i>Barbronia arcana</i>	Mexico	DQ235598	INSd
<i>Barbronia gwalagwalensis</i>	South Africa	AY786455	INSd
<i>Mimobdella japonica</i>	Japan	AB679658	INSd
<i>Mimobdella japonica</i>	Japan	LC090771	INSd
<i>Odontobdella blanchardi</i>	Japan	AB675016	INSd
<i>Odontobdella gaowangjiensis</i>	China	PP510626	INSd
<i>Odontobdella gaowangjiensis</i>	China	PP510627	INSd
<i>Salifa motokawai</i>	Vietnam	LC029428	INSd
<i>Salifa motokawai</i>	Vietnam	LC029429	INSd
<i>Salifa motokawai</i>	Vietnam	LC029430	INSd
<i>Salifa motokawai</i>	Vietnam	LC029431	INSd
<i>Salifa perspicax</i>	Rwanda	HQ336340	INSd
<i>Salifa perspicax</i>	Rwanda	HQ336341	INSd
<i>Salifa perspicax</i>	Rwanda	HQ336342	INSd
<i>Salifa perspicax</i>	Rwanda	HQ336343	INSd
Outgroup			
<i>Erpobdella dubia</i>	USA	AF116023	INSd
<i>Erpobdella japonica</i>	Japan	AB679654	INSd
<i>Erpobdella mexicana</i>	Mexico	DQ235595	INSd
<i>Erpobdella montezuma</i>	USA	GQ368760	INSd
<i>Erpobdella punctata</i>	Canada	HQ336346	INSd
<i>Gastrostomobdella monticola</i>	Malaysia	AB679656	INSd
<i>Mooreobdella quaternaria</i>	China	OR578866	INSd
<i>Mooreobdella quaternaria</i>	China	OR578893	INSd
<i>Orobdella kanaekoikeae</i>	Japan	LC184559	INSd
<i>Orobdella kanaekoikeae</i>	Japan	LC184562	INSd
<i>Orobdella kawakatsuorum</i>	Japan	AB679704	INSd
<i>Orobdella koikei</i>	Japan	AB679688	INSd

Results and analysis

Taxonomy

Family Salifidae Johansson, 1909

Genus *Shibabdella* Tang & Liu, gen. nov.

<https://zoobank.org/46F36208-84A8-44A5-96EB-DAA5DE54E357>

**Diagnosis.** Non-bloodsucking aquatic leech. Medium-sized, lacking spots and stripes. One myognath at the anterior end of each pharyngeal ridge. Accessory copulatory pores absent. Atrium spherical, without pre-atrial loop. Ovisacs reverse-folded. Gonopores separated by six annuli, with male gonopore in somite XII c<sub>2</sub>/c<sub>3</sub> and female gonopore in somite XII c<sub>11</sub>/c<sub>12</sub>. Nephridiopores: fifteen pairs. Post-anal annuli present.



**Remarks.** *Shibabdella* gen. nov. belongs to the family Salifidae because it lacks a pre-atrial loop and has a myognath at the anteriority in each of three pharyngeal ridges. Compared to the genera *Barbronia* and *Dineta*, which have one pair of accessory copulatory pores and three pairs of eyespots, this new genus lacks accessory copulatory pores and eyespots. Compared to the genera *Odontobdella* and *Sinodontobdella*, which have blocky coiled ovisacs and three pairs of large columnar pharyngeal stylets, *Shibabdella* gen. nov. has reverse-folded ovisacs and lamellar myognaths. Compared to the genus *Salifa*, which has gastropores and a pair of conspicuous genital atrium, this new genus has the nephridiopores and lacks the obvious angle of genital atrium. Moreover, because the ovisacs of *Shibabdella* gen. nov. reach somite XXI and the diameter of caudal sucker is approximately the maximum body width, it is different from ovisacs of *Mimobdella*, which are as long as somite XXII and the diameter of caudal sucker is only half of the maximum body width.

***Shibabdella wulingensis* Tang & Liu, gen. nov. et sp. nov.**  
<https://zoobank.org/14550FD5-D9D9-43BD-BAC9-F05A3ED34FD7>

**Specimen materials.** *Holotype* • HNHY01; adult; body length 49.19 mm, maximum body width 4.37 mm, diameter of oral sucker 1.04 mm, diameter of caudal sucker 4.11 mm; Shibadong Cave, Gaoming Mountain, Shibadong Village, Huayuan County, Hunan Province, China (28°22.81'N, 109°29.49'E, 622.13 m); collected August 18, 2024. *Paratypes* • 10 specimens, HNHY02–HNHY11, Shibadong Cave, Gaoming Mountain, Shibadong Village, Huayuan County, Hunan Province, China; collected between September 2024 and January 2025, respectively.

**Etymology.** The genus name *Shibabdella* gen. nov. is a combination of “Shiba” and “bdella.” The first element represents the Shibadong Cave, the type locality of the type species. The specific name of the type species, “*wulingensis*,” means that the type species was first found in the Wuling Mountains of central China.

**Ecology.** This species usually inhabits still waters of the cave at depths of 0.02–0.16 m. The water bottom is normally covered with 0.02–0.10 m of clay and sandy sediments, as well as bat feces, Edwards’s long-tailed giant rat (*Leopoldamys edwardsi*) feces, and insect larvae.

**Diagnosis.** Body flat and cylindrical, creamy or pinkish–white, without spots and stripes. One full somite consists of nine annuli,  $b_1(c_1=c_2)=b_2(c_3=c_4)<a_2>b_5(c_9=c_{10})>b_6(c_{11}=c_{12})$  in adults, while in juveniles,  $c_1<c_2=b_2(c_3=c_4)<a_2>c_9>c_{10}=b_6(c_{11}=c_{12})$ . Eyespots undetectable. Tubular pharynx, reaching to XIV  $c_1$ – $c_4$ , anteriorly with 3 myognaths. Intestine occupies a total of 22 annuli, with a folded inner wall and a diameter significantly larger than crop. Absence of the obvious angle of genital atrium. Ovisacs strip-shaped, folding in the middle of somite XXI and then reversing the coiling, which extends to somite XIX, the distance from the blind end to the female gonopore about 1/3 of the body length.

Morphological characterization

Body creamy or pinkish-white, without spots or stripes, dorsal and ventral surfaces flattened, dorsal sensillum inconspicuous (Fig. 1A, B). Adult body reaching 55 mm in length, juvenile body length less than 25 mm (Table 2).

Whole body 27 somites and 187 annuli observed, somites I–VI comprising ten annuli altogether; full somites VII–XXIV containing nine annuli each,  $c_1+c_2+c_3+c_4+a_2+c_9+c_{10}+c_{11}+c_{12}$ ; annuli width in full somites varies at different growth stages,  $a_2>c_1=c_2=c_3=c_4>c_9=c_{10}>c_{11}=c_{12}$  in adults,  $a_2>c_2=c_3=c_4=c_9>c_1=c_{10}=c_{11}=c_{12}$  in juveniles; somite XXV eight annuli,  $b_1(c_1=c_2)<b_2(c_3=c_4)<a_2(b_3=b_4)>a_3(b_5=b_6)$ ; somite XXVI four annuli,  $a_1=a_2>a_3(b_5=b_6)$ ; somite XXVII three annuli,  $a_1=a_2<a_3$ . Anterior ganglionic mass in 9<sup>th</sup> and 10<sup>th</sup> annuli; ganglia VII–XXIV, of each somite, in  $a_2$ ; ganglion XXV in somite XXV  $c_4$  and  $b_3$ ; ganglion XXVI in somite XXVI  $a_2$  and  $b_5$ ; ganglion XXVII in somite XXVII  $a_2$ .

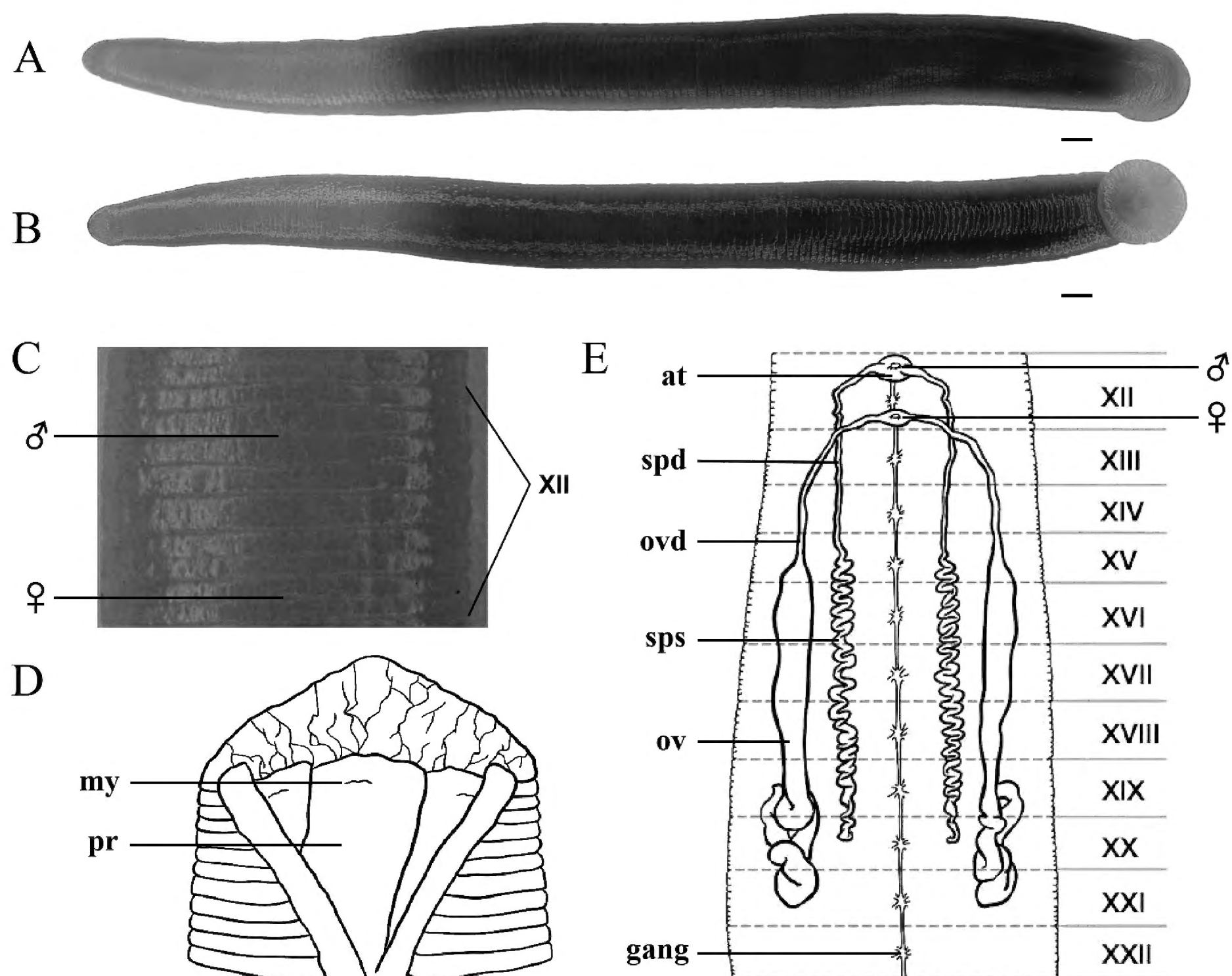
**Table 2.** Morphological measurements (mean ± SD, mm) of the cavernicolous aquatic leech *Shibabdella wulingensis* gen. nov. et sp. nov. in this study.

Measures	Juvenile (N = 5)	Adult (N = 6)
Body length	20.03 ± 1.68	41.37 ± 8.34
Maximum body width	2.62 ± 0.47	4.33 ± 0.51
Head width	1.24 ± 0.18	1.89 ± 0.21
Diameter of oral sucker	0.87 ± 0.19	1.37 ± 0.35
Diameter of caudal sucker	2.56 ± 0.46	4.03 ± 0.33

Male gonopore conspicuous, ventral, in center of somite XII  $c_2/c_3$  (57<sup>th</sup>/58<sup>th</sup> annuli) annular furrow. Female gonopore small, ventral, in center of somite XII  $c_{11}/c_{12}$  (63<sup>rd</sup>/64<sup>th</sup> annuli) annular furrow. Gonopores separated by six annuli (Fig. 1C). Accessory copulatory pores absent.

Eyespots undetected. Oral cavity opening at the base of oral sucker, post-oral annuli after 6<sup>th</sup> annuli. Pharynx muscular, long tubular, with three pharyngeal ridges reaching to somite XIV  $c_1$ – $c_4$ , with 3 myognaths anteriorly (Fig. 1D). Crop tubular, thin-walled, internode constriction. Sphincter connecting crop and intestine in somite XIX  $c_4$ – $a_2$ . Intestine tubular, folded inner wall, reaching to somite XXII  $c_1$ – $c_3$ , significantly larger in diameter than crop, occupying 22 annuli. Rectum terminal opening outward from anus. Anus apparent, curved, dorsal, in center of somite XXVI  $a_1/a_2$  (181<sup>st</sup>/182<sup>nd</sup> annuli). Caudal sucker disc-shaped, slightly dented in the center part. Fifteen pairs of nephridiopores, at the  $c_4$  posterior margin of each somite in X–XXIV.

Atrium spherical in somite XII  $c_2$ – $c_3$ , opening outward through male gonopore. Sperm ducts running alongside the ventral nerve cord, without a pre-atrial loop. Sperm sacs coiling in middle part of somite XV to middle part of somite XX. Strip-shaped ovisacs, in somite XV to somite XXI, with folding in somite XXI and coiling in somites XIX–XXI. Distance from ovisacs terminal to female gonopore occupies about 59 annuli. Oviducts



**Figure 1.** Morphological and anatomical structures of the cavernicolous aquatic leech *Shibabdella wulingensis* gen. nov. et sp. nov. in this study **A.** Dorsal view; **B.** Ventral view (note that the specimen was preserved in 4% paraformaldehyde solution before being photographed); **C.** Ventral view of somite XII; **D.** Ventral view of oral cavity; **E.** Ventral view of reproductive system. Abbreviations: at—atrium; gang—ganglion; my—myognath; ov—ovicel; ovd—oviduct; pr—pharynx ridge; spd—sperm duct; sps—sperm sac; ♂—male gonopore; ♀—female gonopore.

ventrally converging in somite XII  $c_{11}$ – $c_{12}$ , directly descending to female gonopore (Fig. 1E).

### Molecular identification

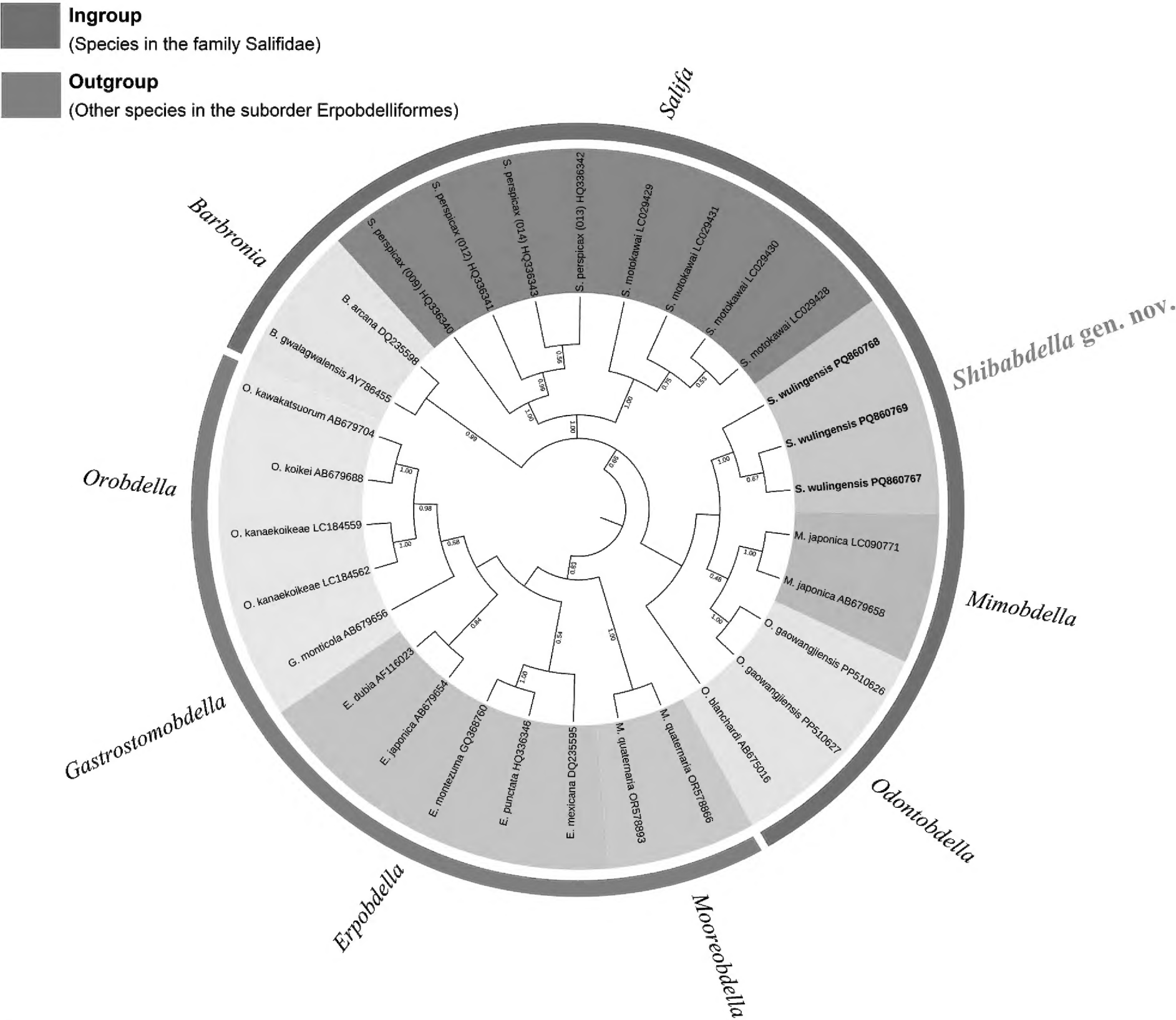
The ingroup of the phylogenetic tree consisted of species from the family Salifidae, while the outgroup included species from *Erpobdella*, *Mooreobdella*, *Gastrostomobdella*, and *Orobdella*, all belonging to the suborder *Erpobdelliformes* (Fig. 2). Nodes with support values greater than 0.45 were labeled. Phylogenetic analysis of the mtDNA COI gene showed that the cavernicolous aquatic leeches collected in this study were positioned within the evolutionary clade of Salifidae species. However, they were distinct from other known genera, forming a separate and well-supported clade. Based on genetic distance analysis, the average genetic distances between *Shibabdella wulingensis* gen. nov. et sp. nov. and species of *Salifa*, *Odontobdella*, and *Mimobdella* were 23.75%, 20.97%, and 20.87%, respectively. The closest average genetic distance was with species of *Barbronia*, at 19.01% (Table 3).

### Discussion

According to phylogenetic analysis, our results were similar to the topological structure proposed by Nakano et al. (2018). Salifidae is the sister group to the family *Erpobdellidae* represented by *Erpobdella* and *Mooreobdella*, with *Gastrostomobdella* and *Orobdella* all belonging to the suborder *Erpobdelliformes*. Moreover, this phylogenetic tree strongly supported (bootstrap = 1.00) monophyly of the *Shibabdella* gen. nov. The genetic distances between the aquatic leech and other species of the family Salifidae have also reached the level of intergeneric differentiation (Hebert et al. 2003).

### Behavior and ecology of *Shibabdella* gen. nov.

Normally, aquatic leeches present a variety of distinguishable behaviors through combinations of lengthening, shortening, and bending of the body, and there are five common types: local bending, systemic shortening, swimming, crawling, and feeding, with swimming and



**Figure 2.** Phylogenetic tree constructed using the maximum-likelihood method based on mtDNA COI gene sequences. Numbers on branches indicate bootstrap support values.

crawling being their main marching motion patterns (Kristan et al. 2005).

During swimming, *Shibabdella wulingensis* gen. nov. et sp. nov. makes an S-shaped wave-like swing, with the body fluctuating dorsoventrally and transmitting a contraction wave from front to back, thus propelling the body forward, and its flattened shape helps to reduce the resistance during swimming. In addition, this species can roll over on the basis of wave-like oscillations in order to change the direction of advancement. It is worth noting that most aquatic leeches exhibit breathable behavior similar to “swimming in place” (Sawyer 1986), and *Shibabdella wulingensis* gen. nov. et sp. nov. is no exception, which is mainly characterized by the caudal sucker being fixed on the attachment and the rest of the body oscillating in a localized wave-like manner, and the fluctuation frequency of the body during the ventilation process is usually slower and more irregular than that of swimming. During movement, this species mainly adopts inchworm-crawling and occasionally worm-crawling

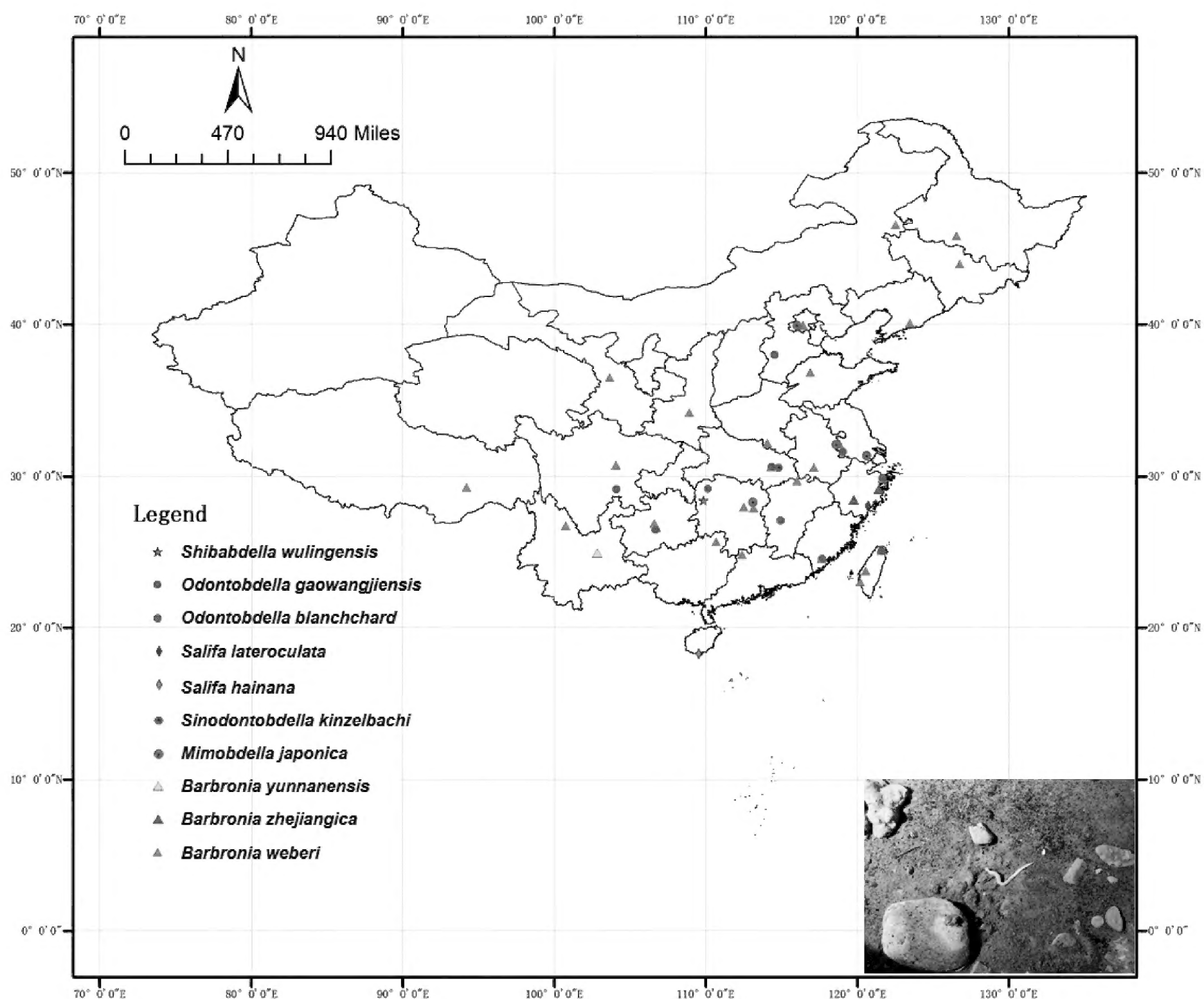
on horizontal surfaces, and worm-crawling is also usually the way for the aquatic leeches to crawl and move on unstable substrates (such as overly loose sediment), showing that *Shibabdella wulingensis* gen. nov. et sp. nov. also adjust their movement control strategies according to changes in the external environment (Sawyer 1986; Kristan et al. 2005; Friesen and Kristan 2007).

This new species inhabits waters with a temperature of 15.8–17.1 °C and a pH of 8.16–8.57. It is often active on the surface of clay sediments or hidden in mud and sand and occasionally adsorbs on rocks in the water with the help of the caudal sucker. Shibadong Cave is located in the interior of Gaoming Mountain, and there is always some water seeping into the caves all year round; thus, the amount of water in the depths of the caves is basically stable. So far, we have only found that this species is active in the still water on the ground of the cave, and any specimens have not been taken from the waters continuously changing due to water dripping or running water movement. The main reason why *Shibabdella wulingensis* gen. nov. et sp. nov. inhabits still water may be that



**Table 3.** Genetic distances between the cavernicolous aquatic leech specimens and other related species in the Erpobdelliformes based on mtDNA COI gene sequences.

	No. of GenBank	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
<i>Shibabdella wulingensis</i> sp. nov.	PQ860767																		
	PQ860768	0.015																	
	PQ860769	0.006	0.009																
<i>Salifa motokawai</i>	LC029428	0.239	0.237	0.233															
	LC029429	0.237	0.235	0.231	0.002														
	LC029430	0.239	0.237	0.233	0.001	0.001													
	LC029431	0.239	0.237	0.233	0.001	0.001	0.000												
<i>Salifa perspicax</i>	HQ336340	0.235	0.239	0.235	0.168	0.166	0.168	0.168											
	HQ336341	0.237	0.241	0.237	0.171	0.169	0.170	0.170	0.041										
	HQ336342	0.241	0.249	0.245	0.176	0.178	0.176	0.176	0.036	0.016									
	HQ336343	0.233	0.241	0.237	0.172	0.174	0.172	0.172	0.036	0.016	0.006								
<i>Odontobdella gaowangjiensis</i>	PP510627	0.212	0.210	0.207	0.238	0.238	0.236	0.236	0.242	0.250	0.254	0.246							
	PP510626	0.210	0.207	0.205	0.239	0.239	0.237	0.237	0.241	0.249	0.253	0.245	0.003						
	AB675016	0.212	0.215	0.209	0.234	0.230	0.232	0.232	0.233	0.226	0.230	0.230	0.221	0.219					
<i>Odontobdella blanchardi</i>	DQ235598	0.198	0.193	0.196	0.240	0.237	0.239	0.239	0.210	0.215	0.223	0.219	0.209	0.206	0.220				
<i>Barbronia arcana</i>	AY786455	0.184	0.184	0.186	0.210	0.207	0.210	0.210	0.197	0.198	0.203	0.200	0.180	0.180	0.216	0.073			
<i>Mimobdella japonica</i>	AB679658	0.207	0.209	0.210	0.222	0.221	0.220	0.220	0.258	0.267	0.274	0.270	0.217	0.216	0.218	0.200	0.190		
	LC090771	0.207	0.209	0.210	0.222	0.222	0.221	0.221	0.258	0.267	0.274	0.270	0.217	0.216	0.218	0.200	0.190	0.000	
	AB679704	0.196	0.204	0.204	0.232	0.231	0.232	0.232	0.240	0.231	0.231	0.227	0.246	0.242	0.257	0.201	0.194	0.245	0.245
<i>Orobdella kawakatsuorum</i>	AB679688	0.197	0.198	0.202	0.240	0.238	0.239	0.239	0.240	0.235	0.241	0.241	0.252	0.249	0.238	0.218	0.205	0.249	0.249
<i>Orobdella koikei</i>	LC184559	0.211	0.207	0.210	0.201	0.199	0.200	0.200	0.229	0.232	0.234	0.232	0.222	0.221	0.208	0.194	0.196	0.230	0.230
<i>Orobdella kanaekoikeae</i>	LC184562	0.213	0.210	0.212	0.204	0.201	0.202	0.202	0.229	0.232	0.234	0.232	0.224	0.223	0.208	0.196	0.199	0.231	0.231
<i>Erpobdella dubia</i>	AF116023	0.227	0.235	0.229	0.238	0.235	0.237	0.237	0.235	0.244	0.250	0.246	0.245	0.246	0.226	0.188	0.171	0.215	0.215
<i>Erpobdella japonica</i>	AB679654	0.205	0.218	0.215	0.222	0.223	0.222	0.222	0.257	0.257	0.262	0.258	0.249	0.243	0.209	0.208	0.203	0.210	0.210
<i>Erpobdella mexicana</i>	DQ235595	0.183	0.193	0.191	0.207	0.205	0.207	0.207	0.237	0.238	0.242	0.233	0.225	0.227	0.246	0.182	0.162	0.236	0.236
<i>Erpobdella montezuma</i>	GQ368760	0.223	0.232	0.227	0.230	0.225	0.227	0.227	0.231	0.239	0.239	0.239	0.242	0.245	0.256	0.203	0.197	0.242	0.242
<i>Erpobdella punctata</i>	HQ336346	0.251	0.265	0.258	0.228	0.227	0.224	0.224	0.236	0.253	0.250	0.250	0.233	0.231	0.281	0.212	0.228	0.269	0.269
<i>Mooreobdella quaternaria</i>	OR578893	0.222	0.232	0.230	0.230	0.231	0.229	0.229	0.227	0.237	0.231	0.231	0.239	0.238	0.237	0.188	0.162	0.262	0.262
	OR578866	0.228	0.228	0.226	0.217	0.219	0.217	0.217	0.223	0.225	0.223	0.223	0.248	0.249	0.227	0.177	0.154	0.255	0.255
<i>Gastrostomobdella monticola</i>	AB679656	0.224	0.228	0.225	0.240	0.239	0.240	0.240	0.269	0.259	0.264	0.260	0.257	0.256	0.253	0.222	0.206	0.242	0.242



**Figure 3.** Geographic distribution map of species within Salifidae in China and habitat photo of *Shibabdella wulingensis* gen. nov. et sp. nov.

the bottom layer of still water is rich in sediment, animal feces, and humus, which makes it easy for insect larvae and microorganisms to grow, and its hiding and food conditions are superior, which is more conducive to the reproduction of this species.

### Distribution and conservation of *Shibabdella*

The species of the family Salifidae are widely distributed in China and have been found in Beijing, Hunan, Hubei, Zhejiang, Taiwan, and other places (Nakano and Lai 2012; Nakano and Lai 2017; Yin et al. 2025). The following is the geographic distribution map of the Salifidae species in China (Fig. 3).

The Wuling Mountains are located at the intersection of three major zoogeographical regions of China, with a warm and humid climate and rich biodiversity. This area also provides suitable environmental conditions for leeches. *Invertebrates of the Southwest Wuling Mountain Region* conducted the first survey report on aquatic leeches in the Wuling Mountains, recording 2 orders, 4 families, 7 genera, and 11 species, including 4 species of *Erpobdelliformes*, all

collected from river waters and paddy fields (Song 1997). However, there has been a lack of research reports on aquatic leeches in the Wuling Mountains over the past 30 years. This is clearly inconsistent with the increasing international attention to biodiversity research and conservation.

Shibadong Cave is named after its eighteen interconnected forks (Niu 2015). The overall length of the cave is about 3 kilometers, and the temperature and humidity inside are relatively stable. It is inhabited by several cave animals such as Myriapoda, Rhaphidophoridae, and Mycetophilidae, which are still to be classified and identified.

However, due to the development of a tourism project a few years ago, most of the cave has been modified with steps and guardrails, and a large amount of construction waste and domestic garbage has been left behind. Artificial damage and man-made pollution in the cave are serious, and the aquatic habitat faces risks of being filled in or drying up naturally. Therefore, how to protect cave animal resources has become an urgent issue that must be addressed alongside tourism development.

We suggest that the relevant departments develop and implement a comprehensive plan for cave animal diversity protection before Shibadong Cave is officially opened



to the public. Such planning is essential to effectively protect *Shibabdella wulingensis* gen. nov. et sp. nov., along with other rare cave-dwelling animal resources, enabling Shibadong Cave to become a model for both cave tourism development and cave biodiversity conservation.

## Funding

This project was funded by the National Natural Science Foundation of China (32160241) and the Hunan Province Undergraduate Training Program for Innovation and Entrepreneurship (S202410531019).

## Acknowledgments

We would like to express our gratitude to Associate Professor You-Xiang Zhang, Dr. Xing-Long Huang, and Dr. Xiao-Chen Yang, as well as many helpful students from the College of Biology and Environmental Sciences of Jishou University, for their support and assistance during the course of laboratory research. In particular, we are truly grateful to Prof. Parin Jirapatrasilp, Pavel Stoev, and Thomas von Rintelen, as well as the anonymous reviewers and editors, for their careful and meticulous reviews.

## References

- Apakupakul K, Siddall ME, Bureson EM (1999) Higher level relationships of leeches (Annelida, Clitellata, Euhirudinea) based on morphology and gene sequences. *Molecular Phylogenetics and Evolution* 12(3): 350–359. <https://doi.org/10.1006/mpev.1999.0639>
- Ewers WH (1974) *Trypanosoma aunawa* sp. n. from an insectivorous bat, *Miniopterus tristis*, in New Guinea, which may be transmitted by a leech. *The Journal of Parasitology* 60: 172–178. <https://pubmed.ncbi.nlm.nih.gov/4814789/>
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299. <https://api.semanticscholar.org/CorpusID:13287260>
- Friesen WO, Kristan WB (2007) Leech locomotion: Swimming, crawling, and decisions. *Current Opinion in Neurobiology* 17: 704–711. <https://doi.org/10.1016/j.conb.2008.01.006>
- Hebert PD, Ratnasingham S, deWaard JR (2003) Barcoding animal life: Cytochrome *c* oxidase subunit 1 divergences among closely related species. *Proceedings. Biological Sciences* 270: S96–S99. <https://doi.org/10.1098/rsbl.2003.0025>
- Huang TF, Liu ZW, Gong XY, Wu T, Liu H, Deng JX, Zhang YX, Peng QZ, Zhang LB, Liu ZX (2019) Vampire in the darkness: A new genus and species of land leech exclusively bloodsucking cave-dwelling bats from China (Hirudina, Arhynchobdellida, Haemadipsidae). *Zootaxa* 4560(2): 257–272. <https://doi.org/10.11646/zootaxa.4560.2.2>
- Kristan WB, Calabrese RL, Friesen WO (2005) Neuronal control of leech behavior. *Progress in Neurobiology* 76: 279–327. <https://doi.org/10.1016/j.pneurobio.2005.09.004>
- Li TY, Liu YH, Zhang C, Gu H, Cheng Z, Peng J, Feng J, Liu Y (2025) A new species of *Sinospelaeobdella* from China: *Sinospelaeobdella jiangxiensis* sp. n. (Hirudina, Arhynchobdellida, Haemadipsidae). *Animals* 15(8): 1079. <https://doi.org/10.3390/ani15081079>
- Liu LR (1984a) A new species of the genus *Erpobdella* (Hirudina, Erpobdellida). *Acta Zootaxonomica Sinica* 2: 138–142. <https://mall.cnki.net/magazine/magadetail/DWFL198402.htm>
- Liu LR (1984b) A new species of the genus *Haemadipsa* (Hirudina, Haemadipsidae). *Acta Zootaxonomica Sinica* 3: 238–240. <https://mall.cnki.net/magazine/magadetail/DWFL198403.htm>
- Liu ZX (2021) *Cave Biology*. Science Press, Beijing.
- Liu YK, Bai Y, Qiao N, Xu SQ (2011) Research progress on the classification of leeches in China. *China science and technology paper online*. <https://www.paper.edu.cn/releasepaper/content/201103-415>
- Magalhães WF, Hutchings P, Ocegüera-Figueroa A, Martin JP, Schmelz R, Wetzel MJ, Wiklund H, Maciolek NJ, Kawauchi YG, Williams JD (2021) Segmented worms (Phylum Annelida): A celebration of twenty years of progress through Zootaxa and call for action on the taxonomic work that remains. *Zootaxa* 4979(1): 190–211. <https://doi.org/10.11646/zootaxa.4979.1.18>
- Matsumoto K (1976) An introduction to the Japanese groundwater animals with reference to their ecology and hygienic significance. *International Journal of Speleology* 8: 141–155. <https://doi.org/10.5038/1827-806X.8.1.13>
- Nakano T (2012) A new species of *Orobdella* (Hirudinida, Arhynchobdellida, Gastrostomobdellidae) and redescription of *Orobdella kawakatsuorum* from Hokkaido, Japan with the phylogenetic position of the new species. *ZooKeys* (169): 9–30. <https://doi.org/10.3897/zookeys.169.2425>
- Nakano T (2017) A New Species of *Orobdella* (Hirudinida, Arhynchobdellida, Orobdellidae) from Japan Reveals the Function of the *Orobdella* Gastroporal Duct. *Zoological Science* 34(2): 161–172. <https://doi.org/10.2108/zs160167>
- Nakano T, Lai YT (2012) A new species of *Orobdella* (Hirudinida, Arhynchobdellida, Orobdellidae) from Taipei, Taiwan. *ZooKeys* 207(207): 49–63. <https://doi.org/10.3897/zookeys.207.3334>
- Nakano T, Lai YT (2017) A new quadrannulate species of *Orobdella* (Hirudinida, Arhynchobdellida, Orobdellidae) from Pingtung, Taiwan. *Species Diversity* 22: 143–150. <https://doi.org/10.12782/specdiv.22.143>
- Nakano T, Meyer-Rochow VB (2016) First record of *Mimobdella japonica* (Hirudinida, Arhynchobdellida, Salifidae) from Hachijoji Island, Izu Islands, Japan, with a comment on the genetic diversity of the species. *Species Diversity* 21(1): 49–54. <https://doi.org/10.12782/sd.21.1.049>
- Nakano T, Nguyen S (2015) A new predatory leech from Vietnam (Hirudinida, Arhynchobdellida, Salifidae): Its phylogenetic position with comments on the classification of the family. *Invertebrate Systematics* 29: 473–486. <https://doi.org/10.1071/IS15008>
- Nakano T, Ramlah Z, Hikida T (2011) Phylogenetic position of gastrostomobdellid leeches (Hirudinida, Arhynchobdellida, Erpobdelliformes) and a new family for the genus *Orobdella*. *Zoologica Scripta* 41(2): 177–185. <https://doi.org/10.1111/j.1463-6409.2011.00506.x>
- Nakano T, Eto K, Nishikawa K, Hossman MY, Jeratthitikul E (2018) Systematic revision of the Southeast Asian macrophagous leeches, with the description of two new gastrostomobdellid species (Hirudinida, Arhynchobdellida, Erpobdelliformes). *Zoological Journal of the Linnean Society* 184(1): 1–30. <https://doi.org/10.1093/zoolinnean/zlx097>

- Nesemann H (1995) On the morphology and taxonomy of the Asian leeches (Hirudinea, Erpobdellidae, Salifidae). *Acta Zoologica Academiae Scientiarum Hungaricae* 41(3): 165–182. [http://actazool.nhmus.hu/ac41\\_3.html](http://actazool.nhmus.hu/ac41_3.html)
- Niu Z (2015) Shibadong Village and to take targeted measures in poverty alleviation. *Newsletter About Work in Rural Areas*(8): 55–56. <http://crnews.net/70/17020151031073453.html>
- Oceguera-Figueroa A, León-Règagnon V, Siddall ME (2005) Phylogeny and revision of Erpobdelliformes (Annelida, Arhynchobdellida) from Mexico based on nuclear and mitochondrial gene sequences. *Filogenia y revisión de los Erpobdelliformes (Annelida, Arhynchobdellida) de México, con base en secuencias de ADN nuclear y mitocondrial*. *Revista Mexicana de Biodiversidad* 76(2): 191–198. <https://doi.org/10.22201/ib.20078706e.2005.002.307>
- Oceguera-Figueroa A, Phillips AJ, Pacheco-Chaves B, Reeves WK, Siddall ME (2011) Phylogeny of macrophagous leeches (Hirudinea, Clitellata) based on molecular data and evaluation of the barcoding locus. *Zoologica Scripta* 40(2): 194–203. <https://doi.org/10.1111/j.1463-6409.2010.00465.x>
- Phillips AJ, Siddall ME (2009) Poly-paraphyly of Hirudinidae: Many lineages of medicinal leeches. *BMC Evolutionary Biology* 9: 246. <https://doi.org/10.1186/1471-2148-9-246>
- Sawyer RT (1986) *Leech biology and behaviour*. Clarendon Press, Oxford.
- Seesamut T, Chanabun R, Likhitrakarn N, Siriut W, Srisonchai R, Pholyotha A, Sutcharit C, Jeratthitikul E (2023) [May] First record of a cavernous land leech *Sinospelaeobdella cavatuses* (Hirudinea, Haemadipsidae) from Thailand. *Tropical Natural History* 23(7): 213–220. <https://doi.org/10.58837/tnh.23.7.257416>
- Siddall ME, Trontelj P, Utevsky SY, Nkamany M, Macdonald KS (2007) Diverse molecular data demonstrate that commercially available medicinal leeches are not *Hirudo medicinalis*. *Proceedings. Biological Sciences* 274(1617): 1481–1487. <https://doi.org/10.1098/rspb.2007.0248>
- Sket B, Dovč P, Jalžić B, Kerovec M, Kučinić M, Trontelj P (2001) A cave leech (Hirudinea, Erpobdellidae) from Croatia with unique morphological features. *Zoologica Scripta* 30(3): 223–229. <https://doi.org/10.1046/j.1463-6409.2001.00065.x>
- Song DX (1974) Preliminary observations on the living habits of leeches in the paddy fields of Yin County, Zhejiang Province, China. *Current Zoology* 1: 52–60. <https://www.cnki.com.cn/Article/CJFDTotal-BEAR197401005.htm>
- Song DX (1997) *Invertebrates of southwest Wuling Mountain Region*. Science Press, Beijing.
- Song DX, Feng ZQ (1978) *Leeches*. Science Press, Beijing.
- Song DX, Zhang J, Tan EG, Liu LR (1977) Research on several kinds of bloodsucking leeches in China. *Current Zoology* 1: 102–108. <https://www.cnki.com.cn/Article/CJFDTotal-BEAR197701013.htm>
- Sudhir K, Glen S, Michael S, Maxwell S, Sudip S, Koichiro T (2024) Mega12: Molecular evolutionary genetic analysis version 12 for adaptive and green computing. *Molecular Biology and Evolution* 41(12): 1–9. <https://doi.org/10.1093/molbev/msae263>
- Tessler M, Barrio A, Borda E, Rood-Goldman R, Hill M, Siddall ME (2016) Description of a soft-bodied invertebrate with microcomputed tomography and revision of the genus *Chthonobdella* (Hirudinea, Haemadipsidae). *Zoologica Scripta* 45(5): 552–565. <https://doi.org/10.1111/zsc.12165>
- Wang H, Meng FM, Jin SJ, Gao JW, Tong XR, Liu ZC (2022) A new species of medicinal leech in the genus *Hirudo* Linnaeus, 1758 (Hirudiniformes, Hirudinidae) from Tianjin City, China. *ZooKeys* 1095: 83–96. <https://doi.org/10.3897/zookeys.1095.74071>
- Yang T (1996) *The fauna of Hirudinea in China*. Science press, Beijing.
- Yang T, Mo X, Wang DB (2009) A new species of cave bloodsucking leeches was found in the western part of Yunnan Province, China. *Acta Zootaxonomica Sinica* 34(1): 125–129. <https://d.wanfangdata.com.cn/periodical/dwfl200901027>
- Yin ZH, Liao JP, Li LB, Liu ZX, Nakano T (2025) A new species of the non-bloodsucking semiaquatic leech genus *Odontobdella* Oka, 1923 (Erpobdelliformes, Salifidae) from the Wuling Mountain area of Central China. *Zootaxa* 5566(1): 159–172. <https://mapress.com/zt/article/view/53838>